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<p>(21) International Application Number: PCT/US97/14218</p> <p>(22) International Filing Date: 13 August 1997 (13.08.97)</p> <p>(30) Priority Data: 08/698,570 16 August 1996 (16.08.96) US</p> <p>(71) Applicant (for all designated States except US): SUPRATEK PHARMA, INC. [CA/CA]; Suite 700, 275 St. Jacques, Montreal, Quebec H2Y 1M9 (CA).</p> <p>(71)(72) Applicants and Inventors: KABANOV, Alexander V. [RU/US]; 1304 South 126th Street, Omaha, NE 68144 (US). ALAKHOV, Valery Y. [RU/CA]; 48 David Kennedy, Baie-D'Urfe, Quebec H9X 3V4 (CA).</p> <p>(74) Agents: BERNSTEIN, Scott, N. et al.; Mathews, Collins, Shepherd & Gould, P.A., Suite 306, 100 Thanet Circle, Princeton, NJ 08540 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: IMPROVEMENTS IN POLYMER COMPOSITIONS FOR CHEMOTHERAPY AND METHODS OF TREATMENT USING THE SAME</p> <p>(57) Abstract</p> <p>Improved antineoplastic compositions comprising an antineoplastic agent combined with a pluronic and a water-soluble non-toxic homopolymer resulting in a decrease of toxicity and/or an increase in anti-cancer activity, and methods of treatment using such formulations.</p>			

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5 **IMPROVEMENTS IN POLYMER COMPOSITIONS FOR CHEMOTHERAPY AND
METHODS OF TREATMENT USING THE SAME**

The present invention relates to improvements in antineoplastic formulations and methods of treatment using such improved formulations.

BACKGROUND OF THE INVENTION

A variety of antineoplastic agents are presently in use in chemotherapy. See generally 10 *Cuttings Handbook of Pharmacology*, 7th Ed., Chapter 13, Scáky and Barnes. However, because of their often complex structure, antineoplastic agents are known to exhibit low stability in the bloodstream. Often, chemotherapeutic agents are extremely insoluble, and are thus poorly transported across cell membranes. Additionally, the effective amount of 15 antineoplastic agents can be greatly reduced through binding of such agents with plasmoproteins, as well as other non-specific interactions in the bloodstream occurring prior to the agents reaching the target. Multidrug resistance (MDR) is a further complication observed with such chemotherapeutic agents, resulting in host resistance to structurally different antineoplastic agents.

Certain antineoplastic agents currently in use have demonstrated toxicity in patients. It is 20 thus desirable to either {i} decrease toxicity of these compositions, {ii} increase their overall anti-cancer activity, or {iii} both. It is similarly desirable to overcome MDR in patients receiving chemotherapeutic agents.

DETAILED DESCRIPTION OF THE INVENTION

The above difficulties can be overcome by administration of antineoplastic agents 25 incorporated into a composition comprising an amphiphilic block copolymer and a non-toxic water-soluble homopolymer or random copolymer.

The invention thus relates to improved compositions comprising an antineoplastic agent combined with an amphiphilic block copolymer and a water-soluble non-toxic homopolymer or random polymer. It has been found that this combination results in {i} a decrease of

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toxicity, {ii} an increase in anti-cancer activity, or {iii} both. The combinations are further capable of reducing or avoiding MDR in patients suffering from neoplasm.

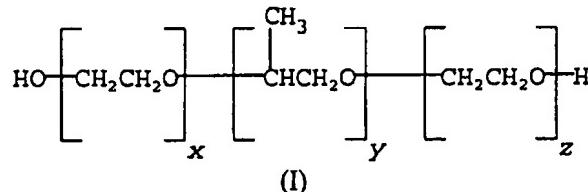
The advantageous properties of the resultant compositions are achieved through the combination of at least one antineoplastic agent, a water-soluble or random non-toxic polymer, and a hydrophobic copolymer, i.e., where the copolymers contain poly(oxypropylene), or POP, content greater than 50 percent.

In one embodiment, the recited homopolymers are water soluble. These polymers can be ionic or capable of being ionically charged in a pH-dependent manner. The preferred molecular mass range for the nontoxic water-soluble homopolymer or random copolymer is 10 from about 0.5×10^3 to 0.5×10^5 . The preferred concentration of these polymers in mixture is from about 1×10^{-4} to about 25% w/v.

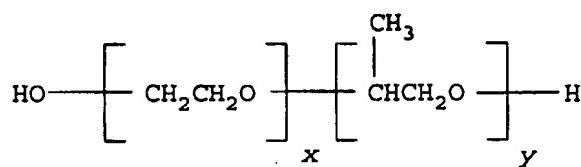
Where a homopolymer is used, the homopolymer is preferably poly(ethylene oxide), or PEO. Where block copolymers are used, the block copolymers are preferably poly(oxyethylene)-poly(oxypropylene) block copolymers. In these POE-POP block copolymers, it has been found that hydrophobe (POP) concentrations greater than 50% are 15 advantageous.

The block copolymers of poly(oxyethylene)-poly(oxypropylene) generally are characterized by one of the following structural formulae:

20



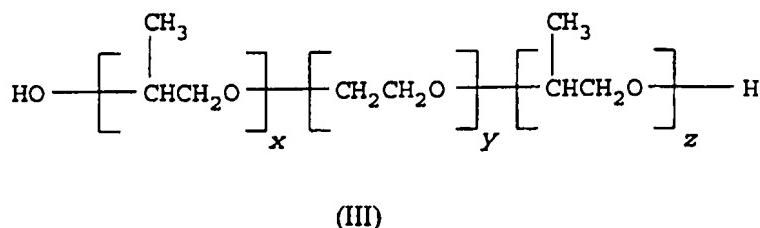
or:



or:

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in which each of x and z , independently of one another, has a value of from about 5 to about 5 100, and y has a value of from about 20 to about 80. Such block copolymers are known. See Schmolka, *Loc. Cit.*, 82(7), 25-30 (1967); Stanton, *Am. Perfumer. Cosmet.*, 72(4), 54-58 (1958); and *Nonionic Surfactants*, Schick, Ed., 300-371 (Dekker, NY, 1967). A number of these copolymers are commercially available under the generic names of "pluronics" and "poloxamers".

10 The hydrophobic/hydrophilic properties of a given block copolymer are dependent upon the ratio of oxypropylene groups to the number of oxyethylene groups. Selecting the ratio of oxypropylene groups to oxyethylene groups involves the use of mixtures of different block copolymers of POE-POP to achieve an optimal balance for a given anti-neoplastic agent, or mixture of anti-neoplastic agents, preserving the optimal particle size.

15 A variety of copolymers are suitable for use in the present invention. The present compositions can utilize, but are not limited to, the following copolymers:

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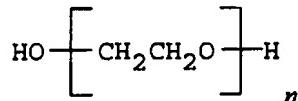
Pluronic	Hydrophobe Weight	Hydrophobe Percentage
L31	950	90%
F35	950	50%
L42	1200	80%
L43	1200	70%
L44	1200	60%
L61	1750	90%
L62	1750	80%
L63	1750	70%
L64	1750	60%
P65	1750	50%
F68	1750	20%
L72	2050	80%
P75	2050	50%
L81	2250	90%
P84	2250	60%
P85	2250	50%
F87	2250	30%
F88	2250	20%
L92	2750	80%
F98	2750	20%
L101	3250	90%
P103	3250	70%
P104	3250	60%
P105	3250	50%
F108	3250	20%
L121	4000	90%
L122	4000	80%
L123	4000	70%
F127	4000	30%
10R5	1000	50%
10R8	1000	20%
12R3	1200	70%
17R2	1700	80%
17R1	1700	90%
17R2	1700	80%
17R4	1700	60%
17R8	1700	20%
22R4	2200	60%
25R1	2500	90%
25R2	2500	80%
25R4	2500	60%
25R5	2500	50%
25R8	2500	50%
31R1	3100	90%
31R2	3100	80%
31R4	3100	60%

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It has been found that the effectiveness of the block copolymers of the instant invention in enhancing the potency of chemotherapeutic drugs, decreasing toxicity, and/or in reducing or reversing MDR depend upon the hydrophobe percentage and the hydrophobe weight. Overall effectiveness of the compositions has been found to increase with an increase in either hydrophobe weight, hydrophobe percentage, or both. It has been found for example that L61 is more effective than P85 which in turn is more effective than F108, which in turn is more effective than F68. One skilled in the art, however, can readily determine the most preferable copolymer based upon the specific circumstances of its intended use.

In accordance with the present invention, the preferred homopolymer is poly(ethylene oxide), or PEO, represented by the formula:



wherein n is such that the molecular mass range of PEO is from about 0.5×10^3 to about 0.5×10^5 . More preferred is from about 1×10^3 to about $.2 \times 10^5$, and most preferred is from about 1.5×10^3 to about $.15 \times 10^5$.

The present application is not, however, limited to the use of PEO. Other water-soluble polymers will work in accordance with the present invention. Suitable water-soluble polymers include without limitation: N-(2-hydroxypropyl)-methacrylamide copolymers, poly(ortho esters), poly(vinyl pyrrolidone), poly(vinyl alcohol), polysaccharides and their derivatives, including dextrane and heparin.

In a most preferred embodiment, the composition comprises doxorubicin as the antineoplastic agent, Pluronic L61 as the block copolymer, and PEO. Other anthracycline antibiotics such as daunorubicin and epirubicin are similarly most preferred. Pluronic L61 is characterized by hydrophobe (POP) content of at least about 90%. It will be appreciated, however, that the present invention is not limited to the recited hydrophobic pluronic polymers.

A variety of antineoplastic agents are suitable for use in accordance with the present invention. These include, but are not limited to, alkaloids such as vinblastine, colchicine, and demecoline; anthracycline antibiotics, including those of the rhodomycin group (e.g., daunorubicin, doxorubicin or epirubicin), those of the mitomycin group

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(e.g., mitomycin C and N-methylmitomycin C), those of the bleomycin group (e.g., bleomycin A₂), and antifolates (including methotrexate, aminopteran, and dideazatetrahydrofolic acid). Mixtures of several of these agents is contemplated within the scope of the invention.

- 5 It will be appreciated that the invention is not directed to the underlying anti-neoplastic activity of these agents, but rather to an improvement in the manifestation of this activity through formulation. The present compositions can be administered parenterally in aqueous formulations, alone or in combination with other therapeutic agents, including other antineoplastic agents, steroids, etc., to a mammal suffering from neoplasm and in need of treatment.
- 10 Such parenteral routes of administration include intramuscular, intrathecal, intraperitoneal, intravenous, and intra-arterial. Isotonic micellar solutions of one or more block copolymers incorporating one or more antineoplastic agents can be used for parenteral administration. Dosages typically are those associated with the specific antineoplastic agent, although the regimen must be titrated to the particular neoplasm, the condition of the patient, and the
- 15 specific response. Thus, the specific dosage will be determined by the attending physician or caretaker, based upon the individual circumstances of the patient. For example, an isotonic micellar solution of daunorubicin is administered to provide about 1 mg of daunorubicin per kg of body weight. In contrast, vinblastine is administered in a similar fashion, but in accordance with conventional usage at lower dosages from about 0.1 to 0.2 mg per kg of
- 20 body weight.

The following examples will serve to further typify the nature of the invention, but should not be construed as a limitation on the scope thereof, which is defined solely by the appended claims.

Example 1

25

Preparation of Three-Component Composition Comprising Pluronic L61, Doxorubicin, and PEO

- Doxorubicin (Sigma, St. Louis) was dissolved at different concentrations in sterilized water (Prep. A). Polyethylene oxide (PEG 8000, Sigma, St. Louis) was then dissolved in
- 30 PBS at a concentration of 10% w/v, and Pluronic L61 (BASF) (0.2% w/v) was added. The mixture obtained was stirred at 4°C until optically transparent (about 45 minutes), (Prep. B).

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Prep. B was then sterilized by filtration through a 0.2 μM filter. Prep. A and Prep. B were then mixed together in equal proportions (Prep. C) and incubated at 37°C for 30 minutes.

Example 2

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In Vitro Evaluation of Anticancer Activity on Lewis Lung Carcinoma (3LL) Cell Line

A highly metastatic clonal Lewis Lung Carcinoma cell subline H-59 (3LL) was used to evaluate cytotoxic activity of Prep. C and compare it with that of doxorubicin. To this end, the cells were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 10 and plated at 2000-3000 cells/well into 96-well microtiter plates. Prep. C in which doxorubicin final concentrations varied from 1 to 10,000 ng/ml was added to the cells and incubated for 2 hours at 37°C and 5% CO₂. The cells were then washed three times with RPMI 1640 and cultured for 4 days. Drug cytotoxicity was determined by a standard XTT assay. To this end, sterile 1 mg/ml XTT solution in RPMI 1640 containing 5 $\mu\text{l}/\text{ml}$ of 1.54 15 $\mu\text{g}/\text{ml}$ phenazine methasulfate solution in sterile PBS was added to the cells (100 $\mu\text{l}/\text{well}$) and incubated for 4 to 16 hours at 37°C and 5% CO₂. The absorbance at λ_{420} was determined using a microplate reader. All the experiments were carried out in triplicate. SEM values were less than 10% (P<0.05). the concentrations of free doxorubicin and doxorubicin in Prep. C producing 50% inhibition of cell growth (IC₅₀) are presented in Table 1.

20

Table 1

Preparation	IC ₅₀ , ng/ml doxorubicin
Prep. C	40
Doxorubicin	110

Example 3

25

Effect of Three Component Composition on Metastasis Development

A highly metastatic clonal subline H59 of Lewis Lung, Carcinoma (3LL-H59) was used as a model. This cell line was established using the *s.c.* grafting of a rare spontaneous 30 lung metastasis detected in a mouse bearing a 3LLc *s.c.* tumor in the axillary region.

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Previous studies showed that this cell line exhibits a good pattern of organ-selective metastasis. These cells were cultured in D-MEM supplemented with 10% FCS at 37°C in a humidified atmosphere with 5% CO₂. After 7 to 10 passes in culture, the cells in their logarithmic growth phase were harvested with trypsin for the following experiments.

5

- Animals.** Female C57BL/6 mice were obtained from Charles River Canada Inc. (St. Constant, Quebec, Canada) and used at 6 to 7 weeks of age. Animals were grouped five per cage with air filter cover under a light (12-h light/dark cycle, light on at 06h00) and temperature-controlled environment (22 ± 1°C). All manipulations of animals were 10 performed under a sterilized laminar. The animals had *ad libitum* access to Purina Mouse Chow (Pro Lab PMH 4018, Trademark of Agway, Syracuse, New York) and water. Animal studies were conducted according to the "Guidelines for Care and Use of Experimental Animals."
- 15 The animals were injected *i.v.* with the cells (5×10^5 cells/animal) and were randomly divided into the four groups (10 animals per group). The animals received the following treatments: 1) Control (isotonic solution), 2) Dox (5.0mg/kg), 3) Dox/L61 [(5.0mg/kg)/(0.25%w/v)], 4) Dox/L61/PEG8000 [(5.0mg/kg)/(0.25%w/v)/(1%w/v)]. Injection volumes were 100ul per animal for all experimental groups. The treatments were performed 3 times at Day 1, Day 4 20 and Day 7 after tumor inoculation.

At Day 16 after tumor inoculation, the animals were sacrificed and subjected to routine metastasis inspection. All organs were routinely screened, although metastatic formations were normally detected only in the lung. Metastatic colonies on the organ surface were 25 enumerated immediately following removal of the organs. Where the number of metastatic nodules on the organ surface was equal to or greater than 50, the animal was considered to have 50 metastasis sites. The results are presented in Table 2. The data are expressed as means ± SEM for the number of metastatic sites and as the percentage of the animals having metastasis for the incidence of metastasis development. Statistical significance was 30 calculated according to the multiple range test of Duncan-Kramer. Analysis of the incidence of metastasis development was done using the Fisher's exact test.

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Table 2

Group	Number of metast. sites on lung periphery ±SEM	Incidence, %
Control	>50	100
Dox	8.90±2.56	100
Dox/L61	0.67±0.55	20
Dox/L61/PEG	0	0

5

Example 4**Effect of Compositions on WBC Count****Cells.** The same as in Example 3.10 **Animals.** The same as in Example 3.

The animals were injected *i.v.* with the cells (5×10^5 cells) and randomly divided into four groups (10 animals per group). The animals received the following treatments: 1) Control (isotonic solution), 2) Dox (5.0mg/kg), 3) Dox/L61 [(5.0mg/kg)/0.25%w/v)], 4) Dox/L61/PEG8000 [((5.0mg/kg)/(0.25%w/v)/(1%w/v)]. The injection volumes were 100ul per animal, for all experimental groups. The treatments were performed 3 times at Day 1, Day 4 and Day 7 after tumor inoculation.

15 At Day 16 after tumor inoculation, the blood samples (20 ul) were collected from the lateral tail vein. Each sample was supplemented with 400 ul of 3% acetic acid, incubated for 20 minutes, and the number 20 of leukocytes was counted (WBC per ml blood).

The results are presented in Table 3. The data were treated by Student's criteria and expressed as means ± SEM.

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Group	WBC count per ml of blood ±SEM
Control	12346±834
Dox	2134±321
Dox/L61	5478±235
Dox/L61/PEG	10358±978

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3 What is claimed:

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6 1. In a pharmaceutical composition comprising an anti-neoplastic agent, the
7 improvement in which said agent is incorporated into micelles of at least one amphiphilic
8 block copolymer and at least one of a water-soluble homopolymer and random
9 copolymer.

10 2. A composition according to claim 1 wherein the block copolymer comprises
11 poly(oxyethylene)-poly(oxypropylene).

12 3. A composition according to claim 2 wherein the poly(oxypropylene) portion of
13 said block copolymer comprises at least 50% by weight of the block copolymer.

14 4. A composition according to claim 1 wherein the molecular mass range for the
15 nontoxic water-soluble homopolymer or random copolymer is from about 0.5×10^3 to
16 about 0.5×10^5 .

17 5. A composition according to claim 1 wherein the nontoxic water-soluble polymer
18 concentration is from about 1×10^{-4} to about 25% w/v.

19 6. A composition according to claim 5 wherein the nontoxic water-soluble polymers
20 are selected from the group consisting of *N*-(2-hydroxypropyl)-methacrylamide
21 copolymers, poly(ortho esters), poly(vinyl pyrrolidone), poly(vinyl alcohol), polysaccha-
22 rides and derivatives thereof.

23 7. A composition according to claim 1 wherein the water- soluble nontoxic
24 homopolymer is poly(ethylene oxide).

25 8. A composition according to claim 1 wherein said anti-neoplastic agent is an
26 anthracycline antibiotic.

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1 9. A composition according to claim 8 wherein the anthracycline antibiotic is
2 selected from the group consisting of doxorubicin, daunorubicin, and epirubicin.

3 10. A composition according to claim 1 wherein said antineoplastic agent is
4 doxorubicin, said block copolymer is Pluronic L61 and said homopolymer is PEO.

5 11. A method of treating a mammal suffering from neoplasm comprising
6 administering to said mammal a therapeutic amount of a composition according to claim
7 I.

INTERNATIONAL SEARCH REPORT

International application No. PCT/US97/14218

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/765, 47/34
US CL : 424/78.08; 514/772.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/78.08; 514/772.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,769,250 A (FORSSEN) 06 September 1998, see columns 5-8.	1-11
Y	VENNE, A. et al. Hypersensitizing Effect of Pluronic L61 on Cytotoxic Activity, Transport, and Subcellular Distribution of Doxorubicin in Multiple Drug-resistant Cells. Cancer Research. 15 August 1996, Vol. 56, pages 3626-3629, see entire document.	1-11
Y	KABANOV, A. V. et al. A New Class of Drug Carriers :Micelles of Poly(oxyethylene)-poly(oxypropylene) Block Copolymers as Microcontainers for Drug Targeting from Blood in Brain. Journal of Controlled Release. 1992, Vol. 22, pages 141-157, see page 147.	1-11

Further documents are listed in the continuation of Box C.

See patent family annex.

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Date of the actual completion of the international search

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